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Effect of Micronisation on the Composition and Properties of the Flour from White, Yellow and Red Maize

Slađana Žilić[1], Vesna Hadži-Tašković Šukalović[2], Marija Milašinović[1], Dragana Ignjatović-Micić[1], Milan Maksimović[3] and Valentina Semenčenko[1]*

[1]Maize Research Institute 'Zemun Polje', Slobodana Bajića 1, RS-11085 Belgrade-Zemun, Serbia

[2]Institute for Multidisciplinary Research, Kneza Višeslava 1, RS-11030 Belgrade, Serbia

[3]Military Medical Academy, Institute of Hygiene, RS-11000 Belgrade, Serbia

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Summary

The process of micronisation, a short time high temperature process that utilizes electromagnetic radiation in the infrared region to rapidly heat materials, is often used to improve storage stability of whole grain flour. In this work the consequences of such temperature treatment on the quality and solubility of proteins, viscosity, content of total phenolics, tocopherols, β-carotene, as well as the antioxidant properties of maize (*Zea mays* L.) flour are presented. For these studies three maize hybrids were used: the semi-flint hybrid ZP 633 with pronounced yellow kernels, ZP Rumenka with dark red pericarp and yellow endosperm, and ZP 551b hybrid which is characterized by white kernels. The process of micronisation did not change the content of crude protein, the amount of albumin, globulin and zein were decreased, while glutelin remained the same or increased after micronisation. As a consequence of thermal effect on maize protein, tryptophan content was significantly decreased. Micronisation had a significant effect on the pasting properties of the selected maize flour. Viscosity of all micronised flour samples increased constantly, but without reaching a peak during heating of the slurry to 95 °C. At 95 °C it was slightly higher, but final viscosity at 50 °C was significantly lower. The micronisation treatment decreased the content of bioactive compounds (tocopherols, β-carotene) naturally present in the raw grains. The whole grain flour from micronised grain, with modified nutritional and technological characteristics, represents a good raw material for production of gluten-free products.

Key words: antioxidants, maize flour, micronisation, protein solubility, viscosity

Introduction

Maize (*Zea mays* L.) ranks as the third most important cereal grain in the world. It is primarily used for food and feed, providing over one half of total calories and total protein demands in developing countries. Whole grain flour and products prepared from it are desirable, mostly due to their taste and nutritional benefits. However, upon milling, the raw whole grain flour results in rapid deterioration, largely due to enzymatic activities, especially those of lipase, lipoxygenase, peroxidase and polyphenoloxidase, which are associated with the lipid component. Stabilized whole grain maize flour with extended storage stability can be obtained by treating the grain with direct heat sufficient to deactivate enzymes (*1,2*). The consequences of the temperature treatment are modified functional properties, improved processing tolerance, improved dough properties and enhanced flavour (*3*). Micronisation is a short time high temperature process that utilizes electromagnetic radiation in the infra-

*Corresponding author; Phone: ++381 11 3756 704; Fax: ++381 11 3754 994; E-mail: szilic@mrizp.rs, sladjana.zilic505@gmail.com

red region to rapidly heat materials (*4*). The process of micronisation is often used to alter functional properties of biomolecules of cereal grain in order to use such grain as an ingredient in food and feed. Micronisation treatment can improve digestibility and rheological characteristics of flour, as well as increase the content of antioxidants, and thus enable wide usage of maize flour in products with extended freshness and storage stability (*4*,*5*).

Protein solubility is an important functional property that affects the utilization and nutritional value of cereal grain. Depending on the solubility in different solvents, proteins from maize grain can be grouped into water-soluble albumins, salt-soluble globulins, alcohol-soluble zeins and alkali-soluble glutelins. Zeins and glutelins constitute storage proteins of maize kernel. The zein fraction accounts for about 50 % of the total endosperm protein. It is characterized by high contents of glutamine, leucine, proline, and is partially devoid of two essential amino acids, lysine and tryptophan, which determine corn protein as nutritionally inadequate (*6*). Zeins can be separated into four distinct subfractions: α, β, γ, and δ (*7*). α-Zein is by far the most abundant, making up to approx. 70 % of the total. It is located in the large central portion of the protein body with β- and γ-zeins on its periphery, which may be the reason for higher thermostability of zein fraction (*8*). Also, zein has some of the properties of wheat gluten, but is not able to form viscoelastic fibrils at room temperature, although it can be made functional at higher temperatures (*9*,*10*). Three glutelin subgroups, denoted G1, G2 and G3, constitute alkali-soluble maize storage proteins. Interpolypeptide disulphide bonds make glutelin poorly soluble (*11*), except for G3-glutelins, which have amino acid compositions similar to those of salt-soluble proteins.

The quality and general acceptability of a cereal food product is reported to be influenced by the physical and chemical properties of the cereal from which it is produced. These properties may be modified through chemical, physical and enzymatic processes to obtain desired functional characteristics (*12*). Viscosity can play an important role in the acceptability of many food products. This technological characteristic of maize flour depends on the influences and interactions between different grain components. However, viscosity is mostly affected by starch type and quantity (*13*). Under the effect of thermal treatments starch granules bind much water, swell and gelatinize, becoming thick and viscous at very low concentrations (*14*).

Maize grain contains a broad variety of phytonutrients, including antioxidants. Antioxidant compounds, such as carotenoids, tocopherols and phenolics play an important role in animal and human nutrition. Carotenoids are a class of fat-soluble antioxidant compounds. Four carotenoid compounds are predominant in maize grain: β-carotene, β-cryptoxanthin, zeaxanthin and lutein (*15*). Although β-carotene has the highest provitamin A activity, it is present in a relatively low concentration in maize kernels. Whole grains are concentrated sources of vitamin E, especially tocotrienols. Vitamin E is the generic term used to describe a family of eight lipid-soluble antioxidants with two types of structures, the tocopherols (α-, β-, γ- and δ-tocopherol) and tocotrienols (α-, β-, γ- and δ-tocotrienol). The most abundant form of vitamin E in maize grain is γ-tocopherol, but α-tocopherol is not uncommon (*16*). Because of the ability to reduce free radicals, vitamin E is an intracellular antioxidant that protects polyunsaturated fatty acids in cellular membranes from oxidative damage (*17*).

The most common phenolic compounds found in whole grains are phenolic acids and flavonoids. Flavonoids specify the colour of the maize pericarp. These compounds are thought to act as free radical terminators, chelators of metal catalyst or singlet oxygen quenchers. Consumption of free radicals and oxidation products may be a risk factor for cancer and cardiovascular disease. Dietary phenolics, due to their antioxidant properties, may have health benefits (*18*,*19*).

Peroxidase activity in raw maize flour can significantly decrease nutritional value and storage stability. Peroxidases (EC 1.11.1.7) are a group of enzymes that can oxidise a large variety of substrates (with the preference for phenolic substances) by using H_2O_2 as oxidizing agent. These enzymes can participate in a great number of oxidative reactions, such as colour change, degradation of chlorophyll and auxins, oxidation of phenols, indol acetic acid and biosynthesis of lignin. Many of these factors are also associated with the flavour, colour, texture and nutritional quality of food (*20*).

The purpose of the present study is to examine the effect of micronisation on the nutritive and rheological characteristics of white, yellow and red maize flour. The quality and solubility of protein, viscosity, total phenolics, tocopherols, β-carotene and their antioxidant properties detected as free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) were investigated. In addition, standard chemical composition of flour obtained from unmicronised and micronised grain was determined. Peroxidase activity was used as the indicator of deterioration of grain enzymatic activities.

Materials and Methods

Maize samples

For these studies the kernels of three maize hybrids developed at the Maize Research Institute, Zemun Polje (MRIZP), Belgrade, Serbia, were used: the semi-flint hybrid ZP 633 with pronounced yellow kernels, ZP Rumenka with dark red pericarp and yellow endosperm and the hybrid ZP 551b, which is characterized by the white colour of kernels. The characteristic differences in grain colour are due to the presence of carotenoids and flavonoids.

Sample preparation

Whole grain flour (integral flour) was produced by milling intact fresh maize kernels in a stone grinder. In order to determine the effect of the process of micronisation on nutritive properties of maize flour, intact kernels of selected ZP hybrids were subjected to the process of micronisation at the temperature of 140 °C for 40 s. Infrared rays were used to roast maize kernels, which

were then flaked under the pressure of rolls. Maize flakes (micronised grain) were ground in the stone grinder to obtain instant flour which was additionally ground into fine powder before use for chemical analyses.

Analytical procedures

Different protein fractions were obtained by successive extractions of defatted flour with a series of solvents (in a ratio of 1:10, by mass per volume) according to the Landry and Moureaux method (*11*), with some modifications. Distilled water, 0.5 M NaCl, 70 % ethanol and 0.0125 M borate buffer, pH=10, containing 5 % sodium dodecyl sulphate (SDS) were used to extract albumin, globulin, α-zein and glutelin (G3-glutelin) fractions, respectively. Extraction of each protein fraction was done by repeated stirring three times for 30 min at 4 °C, followed by centrifugation at 20 000×*g* for 15 min. Protein content was calculated in each fraction from the nitrogen content determined by micro-Kjeldahl method, using 6.25 as the conversion factor. The results are given as percentage of dry mass, as well as percentage of total protein.

Tryptophan content was determined using the colorimetric method of Hernández and Bates (*21*). The colour was developed in the reaction of flour hydrolysate (obtained by overnight digestion with papain solution at 65 °C) with 2 mL of reagent containing 56 mg of Fe^{3+} dissolved in 1 L of glacial acetic acid and 2 mL of 15 M H_2SO_4. After incubation at 65 °C for 15 min, absorbance was read at 560 nm. Tryptophan content was calculated using a standard (calibration) curve, developed with the known amounts of tryptophan, ranging from 0 to 30 μg/mL.

To obtain the pasting curve of various maize flour samples, changes in the apparent viscosity of an aqueous suspension were determined as follows: flour slurry (8 % starch suspension, total mass of 500 g) was heated in a Brabender viscograph at a rate of 1.5 °C/min from 25 to 95 °C, held at the maximum temperature for 30 min, and then cooled at a rate of 1.5 °C/min to 50 °C and held at 50 °C for 10 min. The Brabender viscograph (model PT 100, Brabender Instrument Inc, Duisburg, Germany) was operated according to the official methods for using the Brabender amylograph (*22*).

β-Carotene was extracted from 11 g of flour with three volumes of the solvent petrolether/acetone (70:30, by volume) at 60–70 °C for 1 h. Separation of β-carotene from other carotenoids and pigments was done on the column made of Al_2O_3, Na_2SO_4 and MgO in a ratio of 3:2:1 (by mass). After elution with the mixture petrolether/acetone (97:3), the absorbance of eluate was measured spectrophotometrically at 447 nm. Absorption coefficient of 1 % solution ($\varepsilon_{percent}$=2 500 (g/100 mL)$^{-1}$·cm^{-1}) was used to calculate the content of β-carotene (*23*).

The tocopherol content was determined by the HPLC method (*24*). After the extraction of 0.600 g of sample with 10 volumes of ethyl alcohol containing 0.1 % (by volume) butylated hydroxytoluene (BHT) at 85 °C for 5 min, saponification was done at the same temperature for 10 min by the addition of 0.120 mL of 80 % (by mass per volume) potassium hydroxide. The samples were then cooled in an ice bath and extracted with the addition of 3 mL of hexane and 3 mL of ice-cold distilled water. The pellet was reextracted twice with 3 mL of hexane, washed with 3 mL of distilled water, vortexed, and centrifuged for 10 min. The hexane fraction was dried for 1 h, dissolved in 200 μL of acetonitrile/methanol/methylene chloride mixture (45:20:35, by mass) before loading into the HPLC. Samples were injected into the 5-μm SUPELCOSIL™ LC-Si column (25 cm×4.6 mm diameter, Sigma-Aldrich, Bellefonte, PA, USA). In front of the column was a 5 cm×4.6 mm i.d. guard column packed with 40-μm pellicular silica. The mobile phase consisted of 0.5 % ethyl acetate and 0.5 % acetic acid in hexane at a flow rate of 1.5 mL/min. The fluorescence detector was set at 290 nm excitation and 330 nm emission.

For the DPPH$^{\cdot}$ test, the maize grain extract was prepared by dissolving 0.3 g of flour in 10 mL of 70 % (by volume) acetone. After continuous shaking for 30 min at room temperature, the solution was centrifuged for 20 min at 20 000×*g*. An aliquot of the extract (0.1 mL) was mixed with ethanol DPPH$^{\cdot}$ solution (0.5 mM, 0.25 mL) and acetate buffer (100 mM, pH=5.5, 0.5 mL). After standing for 30 min in the dark, the absorbance was measured at 517 nm against a blank containing absolute ethanol instead of a sample aliquot. The results are expressed as an IC_{50} value that represents the amount of flour (in mg) providing 50 % inhibition of DPPH radicals (*25*).

Total phenolics were determined by the method of Singleton and Rossi (*25*), using the same extract as for the DPPH$^{\cdot}$ test. Briefly, 0.1 mL of extract was mixed with 0.25 mL of Folin reagent, 1.25 mL of 20 % sodium carbonate, and 0.4 mL of deionized water. After standing for 40 min at room temperature, the absorbance was measured at 725 nm. Total phenolic content was calculated as a catechin equivalent (CE) from the calibration curve of catechin standard solutions and expressed as mg of catechin per g of dry mass (dm) (*26*).

For determination of peroxidase (POD) activity, crude homogenate of maize flour (0.5 g) was prepared by constantly stirring maize flour in 10 mL of 0.1 M K-phosphate buffer, pH=7.6 at 4 °C for 1 h. After centrifugation at 20 000×*g* for 15 min, the obtained supernatant was used to determine POD activity. The reaction mixture (1 mL) consisted of 0.1 mM ferulic acid, 1 mM H_2O_2 and an aliquot of the extract containing about 4 mg of the sample in 100 mM K-phosphate buffer, pH=5.5. The initial rate of the absorbance changes at 286 nm (ε=1.68·10^4/(M cm)) was measured. Calculation of enzyme activity was done on the sample dry mass basis (*27*).

The standard chemical methods (*28*) were applied to determine the content of ash, fibre, starch, total proteins and oil.

Statistical analyses

All chemical analyses were performed with three replicates for each of the two micronisation treatments and the obtained results were statistically analyzed. Significant statistical differences of the observed mean values of chemical maize parameters were determined by the Fisher´s least significant differences (LSD) test, after the analysis of variance (ANOVA) for trials set up according to the randomized complete block (RCB) design.

Results and Discussion

The objective of this research was to study the effects of micronisation on the nutritive and technological characteristics of the grain of white, yellow and red maize. In general, any kind of processing is widely believed to reduce the nutritional value of natural foods. On the other hand, thermal processing increases digestibility and bioavailability of nutrients and phytochemicals (*29*).

Standard chemical compositions of raw and micronised maize grains

Basic chemical composition of raw and micronised maize flour is presented in Table 1. Maize flour from unmicronised red grains had the highest content of crude protein (10.35 % of dm). The high temperature treatment during micronisation process resulted in statistically significant decrease of crude protein content in micronised red maize flour, while there were no significant differences in protein levels between the raw and micronised white and yellow maize flour.

The raw white, yellow and red grains contained 67.91, 71.64 and 64.80 % of starch, and 6.55, 5.21 and 5.71 % of oil, respectively. The high temperature of infrared rays did not cause significant reduction in the content of starch and oil.

The content of cellulose and ash ranged from 1.38 to 2.27 % of dm and from 2.66 to 3.53 % of dm, respectively. The effect of high temperature resulted in statistically significant decrease of cellulose content in micronised flour from white and red maize, while the content of ash was decreased in micronised yellow and red maize flour (Table 1).

Protein fractions in raw and micronised maize grains

Protein fractions were isolated according to their solubility in different solutions. The obtained results are presented in Table 2. α-Zein was the dominant protein fraction in raw white, yellow and red maize flour, making 27.1 to 29.5 % of total protein content. The content of G3-glutelins and albumin was 21.2 to 22.3 % and 14.4 to 18.9 % of total protein, respectively. Globulin was the lowest fraction in all unmicronised flour samples (11.0–12.3 % of total protein). Although there were no prominent changes in crude protein content, the amount of albumin, globulin and α-zein decreased, while G3-glutelin remained the same (yellow maize flour) or increased as a result of micronisation. The changes in protein solubility by micronisation suggested that denaturation of proteins occurred in these grains. Thus, globulin content decreased in micronised red maize flour by 58 % compared to its content in raw red maize flour, and by 56 % in white maize flour. Decrease of α-zein fraction ranged from 9 to 40 %. Many studies report that protein solubility is reduced by micronisation (*3*,*30*,*31*). The time of exposure of the grain to infrared heating and grain moisture content had significant effect on protein solubility,

Table 1. Standard chemical composition of maize flour from unmicronised (U) and micronised (M) white, yellow and red grains

Sample	*w*(ash)/%	*w*(protein)/%	*w*(cellulose)/%	*w*(oil)/%	*w*(starch)/%
White grain (U)	3.05^{b}	8.55^{c}	2.19^{a}	6.55^{a}	67.91^{b}
White grain (M)	3.09^{b}	8.48^{c}	1.38^{b}	6.47^{a}	67.21^{b}
Yellow grain (U)	3.17^{b}	8.29^{c}	1.42^{b}	5.21^{c}	71.64^{a}
Yellow grain (M)	2.66^{c}	8.38^{c}	1.39^{b}	5.44^{bc}	71.58^{a}
Red grain (U)	3.53^{a}	10.35^{a}	2.27^{a}	5.71^{bc}	64.80^{c}
Red grain (M)	3.00^{b}	9.92^{b}	1.39^{b}	5.88^{b}	63.54^{c}
$LSD_{0.05}$	0.315	0.091	0.207	0.554	1.231
CV/%	5.53	0.46	6.76	5.18	0.71

The values are given on a dry matter basis; $^{a–c}$values followed by the same letter within a column are not significantly different according to the least significant difference, LSD ($p<0.05$); CV – coefficient of variation

Table 2. The content of albumin, globulin, zein, G3-glutelin and tryptophan in maize flour from unmicronised (U) and micronised (M) white, yellow and red grains. The results are presented as % of dry mass (1) and % of total protein (2)

Sample	Albumin		Globulin		α-Zein		G3-glutelin		Tryptophan
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)
White grain (U)	1.62^{a}	18.9^{a}	1.05^{b}	12.3^{a}	2.52^{b}	29.5^{a}	1.77^{b}	21.5^{d}	0.075^{a}
White grain (M)	0.86^{c}	10.1^{d}	0.46^{e}	5.4^{d}	2.30^{c}	27.1^{b}	2.06^{a}	24.8^{a}	0.039^{bc}
Yellow grain (U)	1.49^{b}	18.1^{b}	0.96^{c}	11.7^{a}	2.23^{d}	27.1^{b}	1.71^{b}	22.3^{b}	0.048^{bc}
Yellow grain (M)	0.83^{c}	10.5^{d}	0.55^{d}	6.6^{c}	1.34^{f}	16.0^{d}	1.80^{b}	22.0^{bc}	0.027^{c}
Red grain (U)	1.49^{b}	14.4^{c}	1.14^{a}	11.0^{ab}	2.84^{a}	27.4^{b}	2.14^{a}	21.2^{d}	0.059^{ab}
Red grain (M)	0.92^{c}	9.4^{d}	0.48^{de}	4.9^{d}	1.91^{e}	19.7^{c}	2.22^{a}	22.6^{b}	0.030^{c}
$LSD_{0.05}$	0.082	0.720	0.082	0.720	0.026	0.634	0.172	0.410	0.026
CV/%	2.66	6.31	3.30	7.16	0.75	2.11	4.93	6.24	4.71

$^{a–f}$values followed by the same letter within a column are not significantly different according to the least significant difference, LSD ($p<0.05$); CV – coefficient of variation

whereby the nature of denaturation in cereal proteins depended on the type of grain (*4*). It is generally recognized that albumins and globulins are more sensitive to heat treatment than prolamins and glutelins. During heat treatment, molecules of albumins and globulins undergo unfolding, thus hydrophobic sites are exposed, resulting in reduced solubility (*32*). Our experiments also point to denaturation of maize prolamin fraction, like that of triticale and barley, although nitrogen solubility in 70 % ethanol was not affected by micronisation for all cereals (*4*). G3-glutelin fraction is true glutelin with high content of two essential amino acids, cysteine and methionine. According to our results, G3-glutelin content was unchanged after micronisation process in yellow and red maize flour, while it was increased by 12 % in white maize flour. Heat treatment of cereals has been reported to significantly increase the G3-glutelins (*33*). It appears that heat treatment prevents polymerization of peptide chains, forming the high molecular mass G3-polypeptide. Heat-induced denaturation of plant proteins may involve aggregation of polypeptide chains through either hydrophobic conformation or disulphide bonding, or both. Such denatured proteins should be solubilized by dissociating and reducing agents such as sodium dodecyl sulphate (SDS) and mercaptoethanol (MCE). Zheng *et al.* (*4*) reported that extraction of residues (after Osborne fractionation) with 0.5 % SDS in borate buffer at pH=10 yielded 15–65 % nitrogen solubility for cereals and 11–56 % nitrogen solubility for legumes. Further extraction with 0.6 % MCE in addition to 0.5 % SDS to borate buffer at pH=10 resulted in 5–40 % additional solubility of cereals, but only 1–2 % additional solubility of legumes, indicating the significance of intermolecular disulphide bonding in cereal proteins.

Content of tryptophan in raw and micronised maize grains

Two essential amino acids, lysine and tryptophan, which humans and animals cannot synthesize, are deficient in maize proteins, making its nutritional value poor (*34*). Since these two amino acids are highly correlated, tryptophan is usually used as a single parameter for evaluating the nutritional quality of the grain protein. According to our results, the highest tryptophan content was detected in raw white maize flour (0.075 %) and the lowest in raw yellow maize flour (0.048 %). In raw red maize flour tryptophan content was 0.059 % (Table 2). The micronisation process resulted in statistically significant decreases of tryptophan content in the white and red flour (44 and 49 %, respectively), in comparison with the raw flour (Table 2).

Viscosity of raw and micronised maize grains

To obtain the pasting curve of various maize flour samples, changes in the apparent viscosity of an aqueous maize flour suspension were determined by using a viscograph. Changes in the apparent viscosity of an aqueous yellow maize flour suspension of raw and micronised samples are presented in Fig 1. Graphs for other samples are not presented because the flour from raw and micronised white and red maize grains had the same or very similar pasting behaviour as the raw and micronised yellow grains, respectively. Raw flour viscosity behaviour during heating from 25 to 95 °C reflects the starch capacity to retain water and swell as the slurry is heated. When flour dispersion is heated, the starch granules retain water and swell. This results in a concomitant increase in apparent viscosity. The viscosity of the paste increases to the point where the number of untreated swollen starch granules is maximal. Peak viscosity is indicative of water-binding capacity. After the temperature increases and the granules absorb as much water as needed to achieve their rupture point, the viscosity decreases to a minimum. This decrease in viscosity is called breakdown. When gelatinized starch cools down, amylose retrogrades, resulting in an increase in viscosity called setback, until gel is formed at the end of the test. In this study, all unmicronised samples produced amylograms with viscosities that were typical for normal (dent) maize grain. Such flour is characterized by moderate pasting viscosity with clear peak viscosity. The micronised samples did not show peak viscosity during heating. Prolonged cooking at 95 °C for 30 min resulted in a constant increase of viscosity in the samples. According to the results (Fig. 1), it is clear that micronisation had a significant effect on pasting properties of the selected maize flour samples. All micronised flour samples had a constant viscosity increase without reaching the peak viscosity during heating of the slurry to 95 °C. Viscosity at 95 °C of all micronised samples was slightly higher, but final viscosity at 50 °C was significantly lower than that of unmicronised samples. These changes in pasting properties of the micronised samples are most probably because of the changes in certain starch and protein properties, first of all, protein solubility. These phenomena could be due to the formation of both, hydrophobic and disulphide bonds in grain during micronisation (*35*). The same authors reported that micronisation at (100±5) °C had detrimental effects on wheat flour gluten functionality, including a decrease in protein solubility and impairment of rheological properties. White *et al.* (*36*) found that little disruption had been caused to wheat starch granules during micronisation. This may have been due to a combination of short micronisation time and the low initial moisture content. However, the literature does not provide any circumstantial or direct evidence that micronisation can in fact 'fracture' starch granules within cereal grains, although such a claim has been made (*37*).

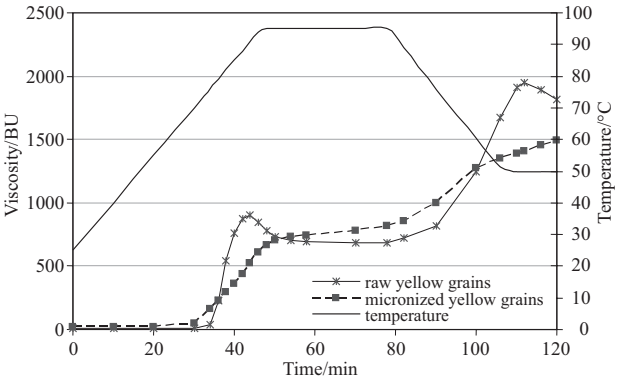

Fig. 1. Brabender amylograph viscosity curves of flour paste of raw and micronized yellow maize grain

Content of β-carotene in raw and micronised maize grains

Provitamin A content in our samples was detected as the content of β-carotene because it has the highest provitamin A activity. The results are presented in Table 3. The highest content of β-carotene (5.43 μg/g) was determined in raw whole grain flour of yellow maize ZP 633. In raw whole grain flour produced from red maize, β-carotene content was lower (2.64 μg/g). The corn genotypes analyzed by Kurilich and Juvik (*38*) contained comparable β-carotene levels to those presented in this study, they ranged from 0.14 to 7.64 μg/g. In our study, white maize flour does not contain β-carotene as was expected, based on previously reported results by Howe and Tanumihardjo (*39*). Quantitative variability in the content of β-carotene of the analyzed maize varieties by Howe and Tanumihardjo (*39*) ranged from undetectable in the white maize grain to 0.77 nmol/g in the yellow, 5.6 nmol/g dm in the orange, and 13.9 nmol/g in the dark orange maize grain. The observed genetic variability suggests profound differences in potential health promotion among genotypes. The ongoing efforts to breed maize for provitamin A have resulted in varieties with 9–17 nmol/g of total provitamin A carotenoids, primarily β-carotene (*38*). Our results show that micronisation heat treatment caused reduction of β-carotene content in micronised yellow maize flour by 23 %. However, high temperature treatment during micronisation process did not result in statistically significant decrease of β-carotene content in micronised red maize flour (Table 3).

Content of total phenolics in raw and micronised maize grains

The content of total phenolics is presented in Table 3, expressed as mg of catechin equivalent (CE) per g of dry mass of flour. The highest content of total phenolics was determined in the flour obtained by milling of both raw and micronised red coloured kernels, which caused red pigmentation in the pericarp (2.76 and 2.81 CE mg/g, respectively), possibly due to the higher amounts of flavonoids. These results are in agreement with the study of Kim *et al.* (*40*), who reported that the level of phenolic compounds in coloured rice seeds was higher than in uncoloured seeds. Phenolic contents were similar in raw and micronised flour of white and yellow maize (Table 3). Although thermal processing has been reported to result in an increase of total phenolic content (*41*), such changes were not detected in the flour obtained from micronised grains in this study. We suggest that short temperature treatment used for micronisation was insufficient to oxidize and polymerize phenolics, which, according to Randhir *et al.* (*41*), improve their antioxidant activity.

Table 3. The content of β-carotene, total phenolics and DPPH· scavenging activity of maize flour from unmicronised (U) and micronised (M) white, yellow and red grains

Sample	γ(β-carotene)	γ(total phenolics, CE)	DPPH-scavenging activity, IC_{50}
	μg/g	mg/g	mg
White grain (U)	n.d.[d]	1.92[b]	5.39[a]
White grain (M)	n.d.[d]	1.92[b]	3.30[b]
Yellow grain (U)	5.43[a]	1.78[b]	5.81[a]
Yellow grain (M)	4.18[b]	1.80[b]	5.37[a]
Red grain (U)	2.64[c]	2.76[a]	3.27[b]
Red grain (M)	2.42[c]	2.81[a]	2.34[c]
$LSD_{0.05}$	0.229	0.025	0.521
CV/%	3.60	5.29	4.72

The values are given on a dry matter basis; [a–c]values followed by the same letter within a column are not significantly different according to the least significant difference, LSD ($p<0.05$); CV – coefficient of variation; CE – catechin equivalent

DPPH· scavenging activity in raw and micronised maize grains

The antioxidant capacity was measured as the DPPH· scavenging activity. An IC_{50} value refers to the quantity of flour (in mg of dry mass, dm) at which DPPH radicals were scavenged by 50 %. Our results are in agreement with earlier findings of Adom *et al.* (*42*) that total phenolic content strongly correlates with total antioxidant activity. The IC_{50} values were higher in yellow and white maize flour, indicating lower antioxidative activity in such flour. Red maize flour, due to the presence of flavonoids, exhibited about 64 and 77 % better scavenging activities than white and yellow maize flour, respectively (Table 3). The process of micronisation improved antioxidant properties of white and red maize flour (Table 3), although it was not accompanied by the increase of total phenolic content. Some of Maillard reaction products derived during the micronisation could be a reason for the increased antioxidant activity in the flour, because some of them are also known to exhibit antioxidant activity (*43*). Nevertheless, our results are in agreement with earlier findings that thermal processing increases antioxidant activity (*44*).

Content of tocopherols in raw and micronised maize grains

The sum of α-tocopherol and γ+β-tocopherol was the highest in raw yellow grain with 64.66 μg/g, followed by white (57.75 μg/g) and red (53.50 μg/g) grain. γ+β--Tocopherol were the dominant form in all samples. The high variability of α-tocopherol content, like that of β--carotene, was observed among the investigated genotypes. The highest α-tocopherol content was estimated in raw whole flour made from yellow maize (20.93 μg/g), significantly lower in red (13.66 μg/g), and hardly detectable in white maize flour (0.58 μg/g). According to Tadmor *et al.* (*45*) the total tocopherol content in maize inbred lines ranged between 65 and 90 mg/g of fresh mass. The amounts of α-tocopherol varied from less than 10 to more than 50 %. In our study, the high temperature of infrared rays caused a reduction in the content of all tocopherol forms. The content of α-tocopherol was lower by 22 and 25 % in micronised yellow and red

flour, respectively. The very low content in white flour was not changed. The content of γ+β-tocopherol was more influenced; about 44 % was destroyed in micronised white and red, and about 33 % in yellow maize flour. All the results are given in Table 4. Barrera-Arellano *et al.* (*46*) reported that the loss of tocopherols at frying temperatures depended on the degree of unsaturation of the lipid substrate, with α-tocopherol being the least stable among the four natural tocopherols. Wyatt *et al.* (*47*) reported the value for total tocopherol content in Mexican corn to be 80.6 μg/g, with relatively high content of γ-tocopherol of 58.0 μg/g, and 22.6 μg/g of α-tocopherol. After thermal treatment, the amount of γ- and α-tocopherol decreased to 32.6 and 9.6 μg/g, respectively.

Table 4. Tocopherol content of maize flour from unmicronised (U) and micronised (M) white, yellow and red grains

Sample	γ(α-tocopherol)	γ(γ+β-tocopherol)	γ(total)
	μg/g	μg/g	μg/g
White grain (U)	0.58^{e}	57.17^{a}	57.75^{b}
White grain (M)	0.58^{e}	32.14^{d}	32.72^{f}
Yellow grain (U)	20.93^{a}	43.73^{b}	64.66^{a}
Yellow grain (M)	16.34^{b}	29.20^{e}	45.54^{d}
Red grain (U)	13.66^{c}	39.84^{c}	53.50^{c}
Red grain (M)	10.26^{d}	23.09^{f}	33.35^{e}
$LSD_{0.05}$	0.081	0.141	0.351
CV/%	0.31	0.14	0.42

The values are given on a dry matter basis; $^{a–e}$values followed by the same letter within a column are not significantly different according to the least significant difference, LSD ($p<0.05$); CV – coefficient of variation

Peroxidase (POD) activity in raw and micronised maize grains

In order to measure the effectiveness of deterioration of the overall enzymatic activity in grains during micronisation, peroxidase activity assay was used, since peroxidase is known to be more tolerant to higher temperatures than other enzymes in cereal grains. Due to this property, peroxidase activity assay is used as a common test in evaluating the adequate heating during oat micronisation (*48*). In our experiments, peroxidase assay was performed with ferulic acid as a natural substrate, because ferulic acid is the main phenolic acid occurring in cell walls. The activity of POD calculated as μmol of ferulic acid oxidized per g of dry mass and min was 0.59, 1.36 and 3.15 of unmicronised white, yellow and red maize, respectively (Fig. 2). The process of micronisation highly decreased peroxidase activity (60 % or more, depending on the sample), and the temperature applied during processing must be sufficient to reduce the activity of many thermolabile enzymes. The improved storage stability of such whole grain flour could

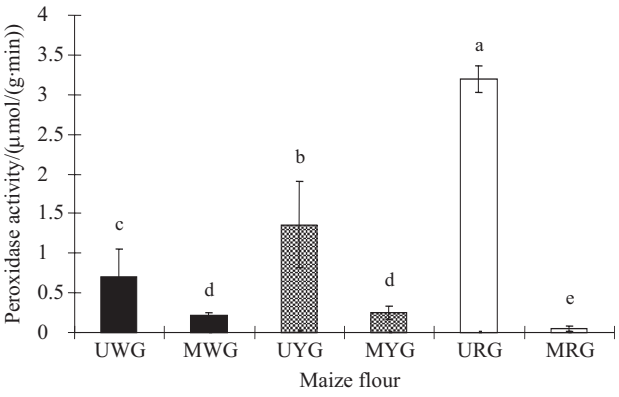

Fig. 2. Peroxidase activity in the flour from unmicronised (U) and micronised (M) white, yellow and red maize grains. UWG – unmicronised white grain, MWG – micronized white grain, UYG – unmicronised yellow grain, MYG – micronized yellow grain, URG – unmicronised red grain, MRG – micronized red grain. Bars with different letters are statistically significantly different ($p<0.05$)

be expected. In addition, being involved in oxidative degradation of carotenoid pigments, the decrease of POD activity should prevent the deterioration of the carotenoid in yellow and red maize flour.

Conclusions

Micronisation of maize (at a temperature of 140 °C for 40 s) did not affect the standard chemical composition of flour from white, yellow and red kernels to a large extent. However, minor decreases of ash, protein and cellulose content were detected in micronised red maize flour. Also, minor decrease of ash was detected in micronised yellow flour and of cellulose in micronised white maize flour. Although changes in crude protein content were minor, the reduced solubility of some fractions indicated the changes in protein structure. Micronisation had negative effect on bioactive compounds (tocopherols, β-carotene) naturally present in the raw grains. This thermal process increased the antioxidant activity and altered the pasting properties of the selected maize flour samples. Due to the reduced POD level in the micronised flour samples, it is expected that they will have increased storage stability. In spite of modified nutritional and technological characteristics, micronised flour represents a good raw material for the production of gluten-free products.

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